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Influence of pyruvate on ammonia metabolism by renal cortical mitochondria

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Influence of pyruvate on ammonia metabolism by renal cortical mitochondria. The effect of the tricarboxylic acid (TCA) cycle precursor, pyruvate, on glutamine metabolism by isolated renal cortical mitochondria was assessed by quantitating its key nitrogen and carbon metabolites. When mitochondria from normal rats were incubated at pH 7.4, pyruvate (2 mM) inhibited ammonia production by almost completely erradicating glutamate deamination and by diminishing glutamine deamidation but to a lesser extent. Alpha KG, citrate, and malate accumulation in the incubation medium were increased dramatically reflecting the increased flux of pyruvate through the TCA cycle; the intramitochondrial concentrations of both Alpha KG and glutamate were increased. Thus, pyruvate primarily inhibits flux through glutamate dehydrogenase as a result either of an increase in Alpha KG concentration and/or a decrease in the redox (NAD/NADH) potential secondary to enhanced flux through the TCA cycle. Glutamine deamidation is secondarily inhibited, presumably due to the increased intramitochondrial concentration of glutamate. Citrate (2 mM) produced changes comparable to those observed with pyruvate. Mitochondria from normal rats incubated at pH 7.0 as well as mitochondria from rats with chronic metabolic acidosis responded to pyruvate in a fashion qualitatively similar to normal mitochondria incubated at pH 7.4. Glutamate deamination was inhibited significantly, but a high rate persisted with chronic acidosis despite the presence of pyruvate. Nevertheless, when glutamine metabolism was contrasted with normal mitochondria incubated at pH 7.4, the response to in vitro incubation in an acid pH as well as to chronic metabolic acidosis was similar quantitatively regardless of whether glutamine alone or in combination with pyruvate was present in the incubation medium.

Influence du pyruvate sur le métabolisme de l'ammoniac par des mitochondries de cortex rénal. L'effet du pyruvate, un précurseur du cycle des acides tricarboxyliques (TCA) sur le métabolisme de la glutamine par des mitochondries isolées de corticale rénale a été étudié en quantifiant ses principaux métabolites azotés et carbonés. Quand des mitochondries de rats normaux étaient incubées à pH 7,4, le pyruvate (2 mM) inhibait la production d'ammoniac en supprimant presque complètement la désamination du glutamate, et en diminuant la désaminationde la glutamine dans une moindre mesure. L'accumulation d'alpha-cétoglutarate, de citrate, et de malate dans le milieu d'incubation était considérablement augmentée, reflétant l'augmentation du flux du pyruvate à travers le cycle des TCA; les concentrations intramitochondriales d'alpha cétoglutarate et de glutamate étaient augmentées. Ainsi le pyruvate inhibe primitivement le flux à travers la glutamate déshydrogénase, ce qui résulte soit d'une augmentation de la concentration d'alpha cétoglutarate, soit (et) d'une diminution du potentiel redox (NAD/NADH) secondaire à la stimulation du flux à travers le cycle des TCA. La désamidation de la glutamine est secondairement inhibée, probablement en raison d'une augmentation de la concentration intramitochondriale de glutamate. Le citrate (2 mM) a entraîné des modifications comparables à celles observées avec le pyruvate. Des mitochondries de rats normaux incubées à pH 7,0, et des mitochondries de rats ayant une acidose métabolique chronique ont répondu au pyruvate de façon qualitativement semblable aux mitochondries normales incubées à pH 7,4. La désamination du glutamate était significativement inhibée, mais une forte activité persistait lors de l'acidose chronique malgré la présence de pyruvate. Toutefois, lorsque le métabolisme de la glutamine était comparé à ce qu'il est dans des mitochondries normales incubées à pH 7,4, la réponse à l'incubation in vitro dans un pH acide, de même que la réponse à l'acidose métabolique chronique, était quantitativement identique que la glutamine soit présente dans le milieu d'incubation seule ou en association avec le pyruvate.

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Experiments both in vivo and in vitro have shown that intermediates of the tricarboxylic acid (TCA) cycle or their precursors can diminish renal ammonia production [1–17] Addition of lactate to the incubation medium increases media glutamate accumulation and diminishes ammonia formation from glutamine by renal slices and tubules [4, 8, 14, 15, 17]. Ammonia production is also decreased when glutamate is used as substrate, which suggests that the primary site of inhibition is the conversion of glutamate to alpha-ketoglutarate (alpha-KG) [15]. This near equilibrium reaction, catalyzed by glutamate dehydrogenase, is thought to be constrained as a result of an increase in alpha-KG concentration and/or a decrease in the redox potential secondary to the increase in metabolism of TCA cycle intermediates [7, 8, 15]. The latter hypothesis is supported by the observation that arsenite, which inhibits the metabolism of alpha-KG, erradicates the inhibitory effect of this substrate on ammoniagenesis as seen in renal slices [6, 7]. Maneuvers that increase dicarboxylate export from isolated mitochondria stimulate ammonia production from glutamine, an effect that is presumed to result from a decrease in alpha-KG concentration [18]. Thus, several lines of evidence point indirectly to alpha-KG and the glutamate dehydrogenase (GDH) reaction as the focal point for the TCA cycle intermediate interaction with glutamine metabolism.

Several observations suggest that pH, per se, as well as chronic acidosis, could modulate ammonia production by altering the metabolism of TCA cycle metabolites. Preuss et al recently reported that the impact of lactate on ammonia production is diminished in renal cortical slices from animals subjected to chronic metabolic acidosis but is unaltered when the medium

Table 1. Effect of pyruvate or citrate on glutamine metabolism by mitochondria from normal rats

	Glutamine	Pyruvate + glutamine (N = 12)		Glutamine	Citrate + glutamine (N = 4)	
	nmoles/min/mg		P	*nmoles/min/mg*		P
Ammonia production	58.5	37.0	<0.01	59.1	44.1	<0.1
Glutamate accumulation	16.1	24.4	<0.01	16.9	23.6	<0.05
Aspartate accumulation	11.0	8.9	<0.01	8.5	7.5	NS
Glutamine deamidation	42.7	35.2	<0.01	42.2	37.5	NS
Glutamate deamination	15.8	1.9	<0.01	16.9	6.5	<0.05
αKG produced[a]	26.8	10.8	<0.01	25.4	14.0	<0.1
αKG accumulation	3.6	20.5	<0.01	3.1	11.0	<0.025
Malate accumulation	4.0	21.4	<0.01	4.8	8.4	NS
Citrate accumulation	0.7	18.7	<0.01	—	—	
Intramitochondrial concentration, *nmoles/mg*						
Glutamate	2.74	5.23	<0.01	—	—	
αKG	0.53	1.13	<0.01	—	—	

[a] αKG was produced from glutamine.

is acidified [15]. Studies with isolated renal cortical mitochondria suggest that a low bicarbonate inhibits pyruvate dehydrogenase activity, which could theoretically alter the effect of pyruvate on ammonia production [19]. Conversely, Simpson and Hager have found recently that a low pH increases mitochondrial uptake of several anions including pyruvate, which theoretically could have the opposite effect [20].

Direct examination of these questions became feasible with the development of methodology using isolated renal cortical mitochondria which permitted an examination of flux rates of glutamine through the intramitochondrial metabolic pathway [21, 22]. These studies were carried out using this technique to test directly the effect of TCA cycle intermediates on mitochondrial ammonia production from glutamine, to identify the metabolic site of inhibition, and to determine if the inhibitory effect is modified by either acute or chronic acidosis.

Methods

Renal cortical mitochondria from male Sprague-Dawley rats were isolated and incubated in vitro as described previously [21–24]. Approximately 2 mg of mitochondrial protein were incubated in 3 ml of medium, which was equilibrated with 95% O_2 to 5% CO_2 and consisted of 100 mM KCl, 25 mM $KHCO_3$, 3.0 mM $MgSO_4$, 4.0 mM ATP, 10.0 mM NaH_2PO_4-Na_2HPO_4, pH 7.4, and 0.8 mM EDTA. Incubations were carried out for 15 min at 37° with 1 mM glutamine either alone or in combination with pyruvate (2 mM) or citrate (2 mM). Mitochondria from normal rats were incubated both at pH 7.4 and 7.0 (achieved by substituting potassium chloride for potassium bicarbonate); mitochondria from rats with chronic metabolic acidosis, induced by drinking 0.28 M NH_4Cl for 1 week, were examined along with pair-fed normal controls.

Analysis of metabolites in the media and intramitochondrially were carried out as described previously [21–24].

Flux rates were calculated in the following fashion:

Glutamine deamidation =
Total NH_3 production − glutamate deamination

Glutamate deamination =
[Total NH_3 − (glutamate + aspartate)] ÷ 2

Alpha-KG produced from glutamine =
Aspartate + glutamate deamination

Statistics were performed using paired and non-paired Student's t tests.

Results

Mitochondria from normal rats incubated at pH 7.4. (Table 1 Figs. 1 and 2). As shown in Figure 1, the addition of 2 mM pyruvate to the incubation medium decreased ammonia production by 21.5 nmoles/min/mg largely due to almost complete elimination of glutamate deamination, which accounts for 13.9 nmoles/min/mg of the decrease. Glutamine deamidation also was inhibited significantly, but to a lesser extent, averaging 7.5 nmoles/min/mg. Glutamate transamination was inhibited slightly by 2.1 nmoles/min/mg. Therefore, as shown in Figure 2, alpha-KG production from glutamine substantially declined by 16.0 nmoles/min/mg. Nevertheless, alpha-KG accumulation in the incubation medium was increased strikingly to 20.5 nmoles/min/mg by the addition of pyruvate and markedly exceeded the rate of alpha-KG production from glutamine (10.8 nmoles/min/mg; $P < 0.01$). Malate and citrate accumulation also increased substantially. These changes in metabolite flux were accompanied by a significant increase in the intramitochondrial concentration of both alpha-KG and glutamate.

In three additional experiments the effect of lower concentrations of pyruvate were examined. At the lowest concentration tested (0.5 mM), pyruvate influenced glutamine metabolism in the same fashion as the studies with 2.0 mM concentrations.

Addition of citrate to the incubation medium resulted in a pattern qualitatively similar to that of pyruvate, but since only four experiments were performed, some changes did not achieve statistical significance (Table 1). Ammonia production decreased in all four studies, largely as a result of a significant diminution in glutamate deamination, with a small decrease in glutamine deamidation which was not significant statistically. The rate of transamination was not altered significantly. Alpha-KG production from glutamine was diminished, but alpha-KG

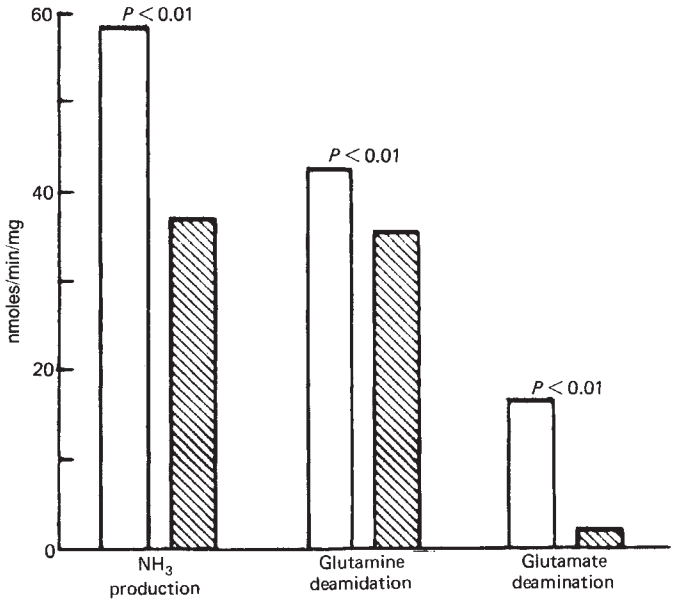

Fig. 1. *Effect of pyruvate (2 mM) on glutamine metabolism by mitochondria from normal rats incubated at pH 7.4.* The *open bars* represent incubations with glutamine alone and the *hatched bars* paired observations with the addition of pyruvate. Pyruvate significantly decreased total ammonia production largely by reducing glutamate deamination. Glutamine deamidation was decreased to a smaller extent.

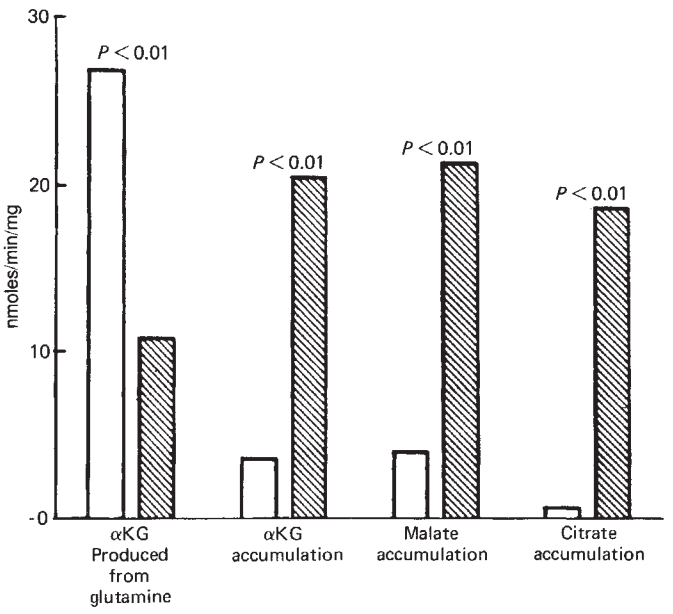

Fig. 2. *Effect of pyruvate (2 mM) on TCA cycle metabolism of mitochondria from normal rats incubated at pH 7.4. Open bars* represent incubations with glutamine alone and *hatched bars* paired observations with pyruvate. Pyruvate significantly reduced alpha-KG formation from glutamine but substantially increased alpha-KG, malate, and citrate accumulation in the incubation medium. The increased formation of TCA cycle intermediates results from conversion of pyruvate to both oxaloacetate and acetyl CoA.

Table 2. Effect of pyruvate on glutamine metabolism by mitochondria from chronically acidotic rats[a]

	Glutamine	Pyruvate + glutamine	
	nmoles/min/mg		*P*
Ammonia production	109	91	<0.01
Glutamate accumulation	32.6	38.5	<0.025
Aspartate accumulation	10.1	12.0	NS
Glutamine deamidation	75.8	70.9	<0.1
Glutamate deamination	33.1	20.4	<0.01
αKG produced[b]	43.2	32.4	<0.01
αKG accumulation	18.6	33.5	<0.025
Malate accumulation	7.2	29.1	<0.01
Citrate accumulation	2.1	19.5	<0.05

[a] $N = 5$.
[b] αKG was produced from glutamine.

accumulation in the incubation medium increased. The increase in malate accumulation was not significant statistically.

Mitochondria from rats with chronic metabolic acidosis (Table 2). Addition of pyruvate to the incubation medium of mitochondria from acidotic rats is given in Table 2. The results qualitatively resemble the response of mitochondria from normal rats, but there are some important quantitative differences.

Despite a markedly higher rate of ammonia production by mitochondria from acidotic rats, pyruvate induced inhibition of total ammonia produced was quantitatively similar to the change with normal mitochondria and averaged 18 nmoles/min/mg. This decrease was largely the result of a reduction in glutamate deamination which averaged 12.7 nmoles/min/mg, with a small decrease in glutamine deamidation (4.9 nmoles/min/mg). It should be noted that a high rate of glutamate deamination persists in the presence of pyruvate, in contrast to the almost total inhibition noted with normal mitochondria. Glutamate transamination was not altered. Therefore, as with normal mitochondria, alpha-KG production from glutamine was decreased significantly. Alpha-KG accumulation in the incubation medium was increased and equivalent to the production rate from glutamine. Pyruvate also substantially increased both malate and citrate accumulation in the medium.

Figure 3 compares the response to chronic acidosis of mitochondria incubated with glutamine alone and mitochondria incubated with both glutamine and pyruvate. Under both conditions the data are represented as the difference between mitochondria from animals with chronic metabolic acidosis and from pair-fed normal controls. Acidosis substantially and significantly increased total ammonia production as a result of an increase in both glutamine deamidation and glutamate deamination, when either glutamine alone or in combination with pyruvate was used as substrate.

Mitochondria from normal rats incubated at pH 7.0. When mitochondria from normal rats were incubated at pH 7.0, addition of pyruvate to the incubation medium decreased ammonia production by 21.4 nmoles/min/mg (Table 3). Similar to studies with mitochondria incubated at a normal pH, the reduction in ammonia production resulted from a substantial decrease in glutamate deamination and accompanying decrease in glutamine deamidation. Glutamate transamination was also significantly reduced, so alpha-KG production from glutamine

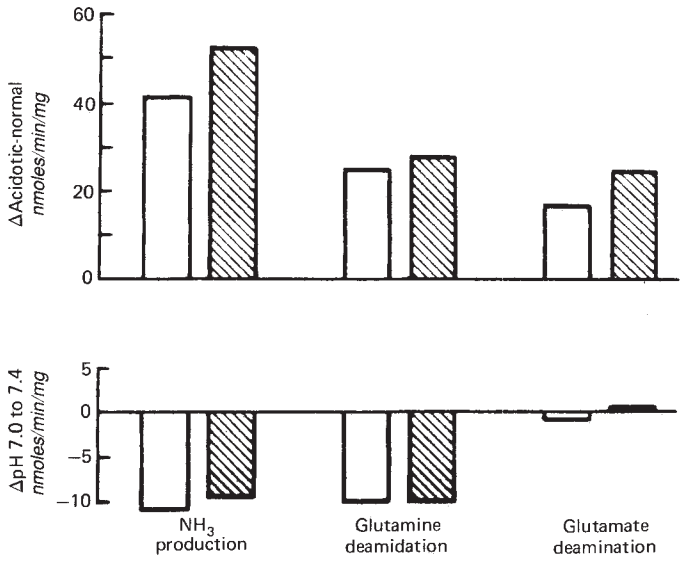

Fig. 3. *The influence of pyruvate on the response of mitochondria to chronic acidosis and to acidification of the incubation medium in vitro.* The *upper panel* depicts the difference between mitochondria from acidotic rats and pair-fed normal controls, while the data in the *lower panel* depict the difference between incubations of normal mitochondria at pH 7.0 and 7.4. *Open bars* represent incubations with glutamine alone reported previously [21]. *Hatched bars* represent incubations with glutamine plus pyruvate. Three studies were carried out with glutamine and pyruvate under conditions of chronic acidosis. Ammonia production ($P < 0.025$), glutamine deamidation ($P < 0.05$) and glutamate deamination ($P < 0.025$) all were increased in a fashion similar to incubations with glutamine alone as substrate. Incubations at a low pH were examined in three studies using glutamine plus pyruvate as substrates. Ammonia production ($P < 0.1$) and glutamine deamidation ($P < 0.05$) appeared to be decreased in a fashion similar to incubations with glutamine alone, while glutamate deamination was not modified.

was depressed substantially by 17.8 nmoles/min/mg. In contrast to incubations at a normal pH, alpha-KG accumulation in the medium was not increased by addition of pyruvate and was negligible in both the absence and presence of pyruvate. Nevertheless, both malate and citrate accumulation increased when pyruvate was added to the incubation medium.

Figure 3 compares the response to a low pH (7.0) by mitochondria incubated with glutamine alone and those incubated with both glutamine and pyruvate. Under both conditions the data are represented as the difference between incubations at pH 7.4. The acidotic pH inhibited ammonia production largely by decreasing glutamine deamidation, when either glutamine alone or in combination with pyruvate was used as substrate. Glutamate deamination was not significantly modified by a low pH with 1 mM glutamine as substrate, in either the presence or absence of pyruvate.

Discussion

These studies with isolated renal cortical mitochondria directly demonstrate that pyruvate inhibits glutamine metabolism primarily by almost complete suppression of glutamate deamination to alpha-KG. This striking inhibition of flux through glutamate dehydrogenase, combined with minimal inhibition of glutamate oxaloacetate transaminase results in an increase in the intramitochondrial concentration of glutamate, which, in turn, apparently inhibits phosphate-dependent glutaminase. However, this secondary effect on glutamine deamidation is much smaller than the inhibition of glutamate deamination.

Table 3. Effect of pyruvate on glutamine metabolism by mitochondria from normal rat incubated at pH 7.0[a]

	Glutamine	Pyruvate + glutamine	
	nmoles/min/mg		*P*
Ammonia production	47.8	26.4	<0.025
Glutamate accumulation	9.0	16.7	<0.01
Aspartate accumulation	7.6	1.1	<0.05
Glutamine deamidation	32.2	22.0	<0.05
Glutamate deamination	15.6	4.3	<0.01
αKG produced[b]	23.2	5.4	<0.025
αKG accumulation	0	0.9	NS
Malate accumulation	6.2	24.2	<0.01
Citrate accumulation	0.6	7.3	<0.1

[a] $N = 3$.
[b] αKG was produced from glutamine.

An alternative explanation for the inhibition of mitochondrial glutamine deamidation by pyruvate is inhibition of mitochondrial glutamine transport by an increased concentration of alpha-KG in the incubation medium. Based on the observation that addition of alpha-KG to the incubation medium inhibits labeled glutamine accumulation by rotenone-inhibited mitochondria, Goldstein has proposed that alpha-KG can directly inhibit mitochondrial glutamine transport [25]. As suggested previously, however, this finding might be explained by reductive amination of alpha-KG to glutamate, which in turn inhibits phosphate-dependent glutaminase (PDG) activity and glutamine transport [26]. Recent studies by Strzelecki and Schoolwerth appear to support this alternative interpretation [27]. Thus, whether or not alpha-KG can directly inhibit glutamine deamidation is controversial. Nevertheless, even if it does, mitochondria incubated at pH 7.0 with pyruvate also demonstrate inhibition of glutamine deamidation but without accumulation of alpha-KG in the incubation medium. These observations with an acid medium, strongly suggest that the effect of pyruvate on glutamine deamidation is not the result of a primary effect of media alpha-KG on the mitochondrial glutamine transporter.

Thus, in addition to the direct demonstration that an alteration in TCA cycle metabolism can inhibit glutamate dehydrogenase, these experiments also indicate that an elevation of intramitochondrial glutamate concentration induced by a physiologic manipulation can inhibit glutamine deamidation. This finding would appear to confirm the hypothesis that regulation of intramitochondrial glutamate concentration can, under physiologic conditions, modulate mitochondrial glutamine metabolism.

The response of mitochondrial glutamine degradation to an alteration in TCA cycle metabolism is not a unique effect of pyruvate, because similar results were obtained with citrate. Citrate clearly inhibited glutamate deamination and appeared to diminish ammonia production. Our results with citrate seem to conflict with the observations by Yu et al, in which no effect on

ammonia production by renal cortical mitochondria from rats and rabbits was observed with 2 mM glutamine as substrate [18]. The reasons for the discrepancy are unclear; however, these investigators analyzed only total ammonia production and the incubation conditions were different from those used in our experiments.

It should be emphasized that although our data clearly show that inhibition of GDH is associated with an increase in intramitochondrial alpha-KG levels, the precise mechanism for the inhibition has not been delineated. Obviously, a direct effect of the increase in alpha-KG concentration is one possibility, but our studies are also consistent with the alternative explanation that the redox state has been altered secondary to accelerated NAD utilization by the added TCA cycle metabolities. Enhanced metabolic flux through the cycle related to both pyruvate carboxylation to oxaloacetate and decarboxylation to acetyl COA was demonstrated by the increased accumulation of malate and citrate in the incubation medium and accumulation of alpha-KG in excess of that produced from the glutamine carbon skeleton.

Another goal of these experiments was to determine whether or not altered acid-base homeostasis would modify the effect of pyruvate on glutamine metabolism. The effect of lactate on glutamine metabolism in renal cortical slices is unaltered when incubations are carried out at pH 7.0 rather than 7.4 [15]. However, studies with isolated mitochondria suggest that a low media bicarbonate concentration inhibits pyruvate metabolism resulting in its decreased utilization and decreased citrate and Alpha KG production [19]. To the contrary, other recent observations suggest that a low pH enhances mitochondrial pyruvate uptake [20]. In addition, Schoolwerth et al have found recently that the effect of a low pH on mitochondrial glutamine metabolism is altered when incubations are carried out with glutamine plus Alpha KG rather than glutamine alone in the incubation medium [28]. Thus, with glutamine alone as substrate a low media pH inhibits ammonia formation, but, when alpha-KG and glutamine are used together as substrates, in vitro acidosis stimulates mitochondrial ammonia formation [23, 28]. In part this stimulation is thought to be secondary to the acceleration of alpha-KG decarboxylation induced by a low pH [22]. Therefore, it seemed important to examine the effect of pyruvate in an acidotic incubation medium. As shown in Table 3, pyruvate inhibited glutamine metabolism with incubations at pH 7.0 in essentially the same fashion as during incubation at pH 7.4. The only notable difference was the lack of increased alpha-KG accumulation in the media when pyruvate was present, presumably because the low pH accelerated Alpha KG conversion to succinate [22]. Although the decrease in alpha-KG accumulation in the media could also be related to a pH-induced inhibition in pyruvate metabolism, this alone would seem insufficient to explain the absence of alpha-KG accumulation, since high rates of malate and citrate accumulation persisted. As shown in Figure 3, the presence of pyruvate did not alter the response to a low pH. Ammonia production appeared to be diminished by a low pH both in the presence and absence of pyruvate, and the major effect was an inhibition of glutamine deamidation. Thus, in apparent contrast to observations with alpha-KG, the presence of the TCA cycle precursor, pyruvate, in the incubation medium does not reverse the depressive effect of a low pH on glutamine metabolism.

Preuss et al recently reported that lactate-induced inhibition of ammonia production was diminished substantially when renal slices from chronically acidotic rather than normal rats were examined [15]. These findings suggested that glutamine metabolism might be altered during the adaptation to acidosis in a fashion which modified the impact of TCA cycle metabolism on the ammonia producing process. Our observations with isolated mitochondria are generally consistent with Preuss et al findings in slices and provide some additional insight into the mechanisms involved. As shown in Table 2, pyruvate suppresses ammonia formation by mitochondria from acidotic rats in the same fashion as with mitochondria from normal animals, that is, by strikingly inhibiting glutamate deamination. In contrast to studies with normals however, a substantial flux of glutamate to alpha-KG persists in the acidotic mitochondria because the basal rate of GDH flux is twice that in normal mitochondria. Thus, the absolute decrements in both glutamate deamination (13 vs. 14 nmoles/min/mg) and total ammonia production 18 vs. 21.5 nmoles/min/mg are remarkably similar in the acidotic and normal mitochondria, but the percentage change in acidosis is dramatically less. In fact, the pattern is similar to observations of Preuss et al in slices, where the absolute magnitude of the change in acidotic and normal slices was also relatively similar. Thus, pyruvate behaves qualitatively in a similar fashion in acidosis, but the specific kinetics of the altered inhibition of GDH requires closer examination. Finally, it should be noted in Figure 3 that the basic mitochondrial response to acidosis is the same in the presence or absence of pyruvate. Ammonia production is stimulated by chronic acidosis as a result of an increased flux through both PDG and GDH.

In summary, our studies demonstrate that the TCA cycle metabolite citrate and precursor, pyruvate, can alter mitochondrial glutamine metabolism by inhibiting GDH. Although TCA cycle metabolism can alter mitochondrial metabolism under normal as well as acute and chronic acidotic conditions, the qualitative response to acid-base deviations does not appear to be modified by the presence or absence of pyruvate.

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